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ORIGINAL ARTICLE

Mitochondrial Diversity and Phylogeographic Patterns of *Gekko gecko* (Squamata: Gekkonidae) in Thailand

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Abstract The Tokay Gecko, *Gekko gecko* (Linnaeus, 1758) is widely distributed in Asia and there have been concerns regarding locally decreasing populations due to overexploitation for traditional Chinese medicine. Previous studies of the genetic relationships of *G. gecko* populations included few populations from Thailand. Here we investigated the phylogeographic patterns of *G. gecko* from different regions in Thailand using mitochondrial cytochrome *b* sequences. Phylogenetic analyses revealed two lineages: one (Lineage A) comprising populations from Laos, Vietnam, and Thailand; and a second (Lineage B) comprising three genetically distinct groups within Thailand alone. Some Thai populations were found to have both lineages represented within them. Highly significant genetic differentiation (F_{ST}) showed geographic population structuring in Lineage B, indicating limited gene flow among groups in Thailand. Although *G. gecko* has a wide distribution and is well adapted to human habitation, the observed genetic structure could potentially be explained by geographic barriers such as mountain ranges. In Lineage A, our study provided primary phylogeographic evidence for lineage mixture that might be a result of human-mediated transport. Future research should include more extensive sampling across the geographic distribution of *G. gecko* and a landscape genetics approach could be applied for conservation planning.

Keywords genetic structure; haplotype, Gekkonidae, mitochondrial DNA, human-mediated transport

1. Introduction

Southeast Asia has a unique geological history that has contributed to high regional biodiversity (Sodhi *et al*., 2004; Woodruff, 2010). To understand the diversification of organisms and historical biogeography of this region, studies on adaptation and evolution of reptiles in response to the geographical and climatic changes are of great interest (e.g., Brown *et al*., 2012; Heinicke *et al*., 2012; Siler *et al*., 2013), particularly studies exploring cryptic diversity of reptiles at both species and population levels (e.g., Brown *et al*., 2012; Grismer *et al*., 2012). Cryptic species are two or more distinct species erroneously classified (and hidden) under a single species name (Bickford *et al*., 2007). In some cases two or more members of a cryptic species complex can occur syntopically, making identification challenging and confounding the interpretation of results from studies based on specimens from these populations (McLeod, 2010; Stuart *et al*., 2006). Moreover, the genetic structure of populations can be affected by human activities such as habitat fragmentation and human-mediated transport (Santos *et al*., 2018; Templeton *et al*., 2001). In these instances, genetic divergence can be used to determine whether individuals and populations belong to the same or separate evolutionary lineages (Ferguson, 2002) and can indicate the level of connectivity among populations (e.g., Blair *et al*., 2013; Dubey *et al*., 2011).

Among vertebrates, reptiles are good models for phylogeographic studies because of their global

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distribution (Pincheira-Donoso *et al*., 2013; Uetz, 2000), and a high degree of genetic divergence (Lin *et al*., 2010). Very few phylogeographic studies of reptiles have been conducted in Thailand. Lukoschek *et al*. (2011) and Saijuntha *et al*. (2017) used mitochondrial DNA (mtDNA) to study the phylogeography of the Mekong Mud Snake (*Enhydris subtaeniatus*) and Blue Crested Lizard (*Calotes mystaceus*), respectively. The results of these studies have suggested that genetic structure of reptile populations in Thailand might be affected by geographic barriers such as mountain ranges and river drainage systems.

The Tokay Gecko *Gekko gecko* (Linnaeus, 1758) belongs to the family Gekkonidae and has a wide distribution in Asia (Rösler, 2005; Smith, 1935; Uetz *et al*., 2018). It has been introduced to many regions of the world and is considered an invasive species in the United States and Brazil (Meshaka *et al*., 1997; Rocha, 2015). The Tokay Gecko has long been heavily exploited for Chinese traditional medicine, and this has led to localized population declines (Bauer, 2009) in parts of Bangladesh, Indonesia, and Thailand (Caillabet, 2013). The taxonomic status of *G. gecko* remains controversial and it is reported to comprise two morphotypes (black-spotted and red-spotted geckos). These two morphotypes occur allopatrically and have high genetic divergence based on mitochondrial and nuclear DNA (Qin *et al*., 2007, 2012; Wang *et al*., 2012, 2013), and different chromosome characteristics (Qin *et al*., 2012). Rösler *et al*. (2011) revalidated the taxonomic status of the black-spotted gecko as *Gekko reevesii* (Gray, 1831) based on morphological, molecular, and zoogeographic evidence. In addition, the two morphotypes have different advertisement calls (Yu *et al*., 2010) and ecological data showed niche differentiation (Zhang *et al*., 2014), corroborating Rösler *et al*. (2011)'s revalidation of *G. reevesii* in China and northern Vietnam. There are currently two recognized subspecies based on morphology, namely *G. gecko gecko* (Linnaeus, 1758), and *G. gecko azhari* Mertens, 1955. *Gekko g. gecko* is widely distributed, ranging from northeast India, southern China, and Southeast Asia, whereas *G. g. azhari* is found only in Bangladesh (Rösler *et al*., 2011; Smith, 1935; Rösler, 2001, 2005). This study considers only samples from outside of Bangladesh and therefore we refer the study taxon (*G. g. gecko*) herein as *G. gecko*.

Although *G. gecko* has a wide distribution range in Asia, phylogeographic analyses of this species from some geographical areas, including Thailand, have been limited (Kongbuntad *et al*., 2016; Qin *et al*., 2007, 2012; Wang *et al*., 2012, 2013; Zhang *et al*., 2006). This study examines the genetic structure of *G. gecko* populations in Thailand. We used mitochondrial cytochrome *b* (cyt *b*) DNA sequence data to assess the mitochondrial DNA diversity and phylogeographic patterns of *G. gecko* populations in Thailand, and compare Thai samples to specimens from Laos, Vietnam, and southern China.

2. Materials and Methods

2.1. Sample collection Field work was conducted from 2010–2018. A total of 114 samples were collected from 17 populations in the residential areas throughout Thailand where *G. gecko* occurs as a human commensal (Table 1; Figure 1). Tail tips of *G. gecko* were clipped, and geckos were returned to where they were captured. Liver tissues were collected from individuals that were kept as voucher specimens. All tail tip and liver tissues were preserved in 95% ethanol and stored at –20 °C for genetic analyses. Voucher specimens were fixed in 10% formalin and subsequently transferred to 70% ethanol. All tissues and voucher specimens were deposited in the herpetological collection, Zoological Museum, Kasetsart University (ZMKU).

2.2. DNA extraction, PCR amplification, and sequencing Total genomic DNA was extracted from tail tip or liver tissues preserved in 95 % ethanol using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. Part of the mitochondrial cyt *b* gene was amplified by polymerase chain reaction (PCR) using the combination of forward and reverse primers from Wang *et al*. (2012) (Table 2). Amplification conditions included initial DNA denaturation at 94°C for 5 min; followed by 35 cycles of 94°C for 1 min, 50°C annealing temperature for 50 s, and 72°C for 1 min; with a final extension at 72°C for 10 min. PCR products were purified using a QIAquick purification kit (Qiagen, Hilden, Germany) and sequenced in both forward and reverse directions using the PCR primers. The sequencing reactions were analyzed on a 3730 DNA Analyzer (Applied Biosystems, CA, USA) by Macrogen Inc. (Seoul, Korea). Sequences were checked by eye, and aligned using MUSCLE implemented in Geneious R11 (Biomatters, Ltd., Auckland, New Zealand). All DNA sequences obtained in this study were deposited in GenBank (Accession numbers MK598399–MK598441).

2.3 Data analyses

Phylogenetic reconstruction

Additional cyt *b* sequences of *G. gecko* from Phonsavan, Laos (GenBank: EF174461, EF640157), Langson,

Table 1 Locality, samples sizes (n), Number of haplotype, MtDNA lineage, and haplotype distribution of *Gekko gecko* used in the present study.

No.	Locality (Province, District)	Code	Latitude	Longitude	Sample size (*n*)	No. of haplotype (Nh)	MtDNA lineage	Haplotype distribution (No. individual)
1	Loei, Phu Ruea	LE	17.422684	101.459448	7	2	B2	H20 (1), H21 (6)
2	Khon Kaen, Waeng Noi	KK	15.810563	102.415894	7	1	B2	H19 (7)
3	Nakhon Ratchasima, Wang Nam Khiao	NR	14.509586	101.931036	19	6	A2, B2	H5 (1), H7 (3), H8 (3), H10 (8), H11 (3), H18 (1)
4	Chanthaburi, Kang Hang Meaw	CT	13.135393	101.97300	4	3	A2	H4 (1), H5 (2), H6 (1)
5	Chonburi, Sri Racha	CB	13.168862	101.087946	1	1	B3	H28 (1)
6	Saraburi, Sao Hai	SR	14.550146	100.847831	17	8	B2	H9 (1), H10 (1), H12 (5), H13 (1), H14 (1), H15 (6), H16 (1), H17 (1)
7	Phitsanulok, Nakhon Thai	PL	17.020493	100.925163	2	2	A1, A2	H2 (1), H5 (1)
8	Uthai Thani, Lan Sak	UT	15.578954	99.413981	4	4	B3	H23 (1), H24 (1), H25 (1), H26 (1)
9	Kanchanaburi, Thong Pha Phum	KB	14.667833	98.598191	7	4	B3	H32 (4), H41 (1), H42 (1), H43 (1)
10	Ratchaburi 1, Mueang	RB1	13.580977	99.831455	8	5	A1, B3	H1 (1), H33 (1), H34 (1), H36 (4), H39 (1)
11	Ratchaburi 2, Suan Phueng	RB2	13.478048	99.252775	3	3	B2, B3	H10 (1), H11 (1), H39 (1)
12	Phetchaburi 1, Cha Am	PB1	12.885258	100.015093	8	3	B3	H37 (1), H38 (1), H40 (6)
13	Phetchaburi 2, Kaeng Krachan	PB2	12.804437	99.574896	3	3	B3	H29 (1), H30 (1), H35 (1)
14	Prachuap Khiri Khan, Bang Saphan	PK	11.213513	99.513924	16	3	B3	H31 (14), H34 (1), H36 (1)
15	Ranong, Mueang	RN	9.853019	98.621854	2	1	B3	H27 (2)
16	Surat Thani, Ban Ta Khun	ST	8.955667	98.805028	2	1	B1	H22 (2)
17	Songkhla, Hat Yai	SK	6.940250	100.254389	1	1	A2	H3 (1)

Vietnam (GenBank: EF640155, EF640156), and Guangxi, China (GenBank: EF174462) were downloaded from GenBank and included in the phylogenetic analyses. *Gekko reveesii* from Guangxi and Yunnan, China (GenBank: EF640150–EF640154, EF640179) and *Gekko vittatus* Houttuyn, 1782 (GenBank: NC008772) were chosen as outgroups. Phylogenetic relationships among haplotypes were estimated using both maximum likelihood (ML) and Bayesian inference (BI). A ML tree was constructed in the IQ-TREE web server (Trifinopoulos *et al*., 2016) using the Bayesian information criterion (BIC). The HKY+F+G4 model was selected as the best-fit model of evolution for all codon positions. A BI analysis was performed using the CIPRES Science Gateway portal V. 3.3 (Miller *et al*., 2015) with default priors. The best-fit substitution model (GTR+I+G) was selected using the Akaike Information Criterion (AIC) as implemented in jModelTest 2.1.10 (Daribba *et al*., 2012). Phylogenetic reconstruction was performed running Metropolis-coupled Markov chain Monte Carlo sampling with four chains for 10 million generations and trees were sampled every 1 000 generations. The first 25% of trees were discarded as burn-in and the 50% majority-rule consensus of the remaining trees was constructed to calculate the posterior probabilities of nodes. Stationarity was checked in Tracer v1.7.1 (Rambaut *et al*., 2018) to ensure that effective sample sizes were above 200 for all parameters.

Genetic diversity and population genetic analyses

Haplotypes were extracted using DnaSP v6.12.06 (Rozas *et al*., 2017). Population genetic diversity among and within sampled populations i.e., haplotype diversity (*h*; Nei, 1987), nucleotide diversity (π; Nei and Tajima, 1981), the numbers of haplotypes (N_h), and the numbers of unique haplotypes (N_u) were calculated using DnaSP v6.12.06. Uncorrected pairwise sequence divergences (*p*-distances) between paired phyloclades were calculated in MEGA v7 (Kumar *et al*., 2018). We constructed a median joining network using Network v4.6.0 (Fluxus Technology Ltd., England) to determine the haplotype relationships and calculated the genetic differentiation among gecko populations as pairwise fixation indices

Table 2 Primers for PCR amplification and sequencing from Wang *et al.* (2012).

Primer	Sequences (5′–3′)
Forward	CACCAAAACCAGTAGTCCGA
Forward	GACCTTCCAACACCATCAAAC
Reverse	CAAGGCCAGTGTATTTGATGT
Reverse	GGGACAAGTAATGGGCACTT
Reverse	GAGCCCCATTTCTGGTTTAC

(F_{ST}) in Arlequin v3.11 (Excoffier *et al.*, 2005). Genetic structure among and within geographic regions was assessed using the Analysis of Molecular Variance (AMOVA) approach implemented in Arlequin.

Population demographic history

The possibility of demographic expansion of *G. gecko* populations in Thailand was tested based on the mismatch distribution of pairwise differences (Rogers and Harpending, 1992) in Arlequin. The Harpending's raggedness index (r) and sum of squared deviations (SSD) were calculated to compare observed and expected distributions under the expansion model. We calculated a neutrality test using Fu's F_s (Fu, 1997) and Tajima's D (Tajima, 1989) in Arlequin with 1 000 permutations. Significant negative values of Fu's F_s and Tajima's D would indicate demographic expansion.

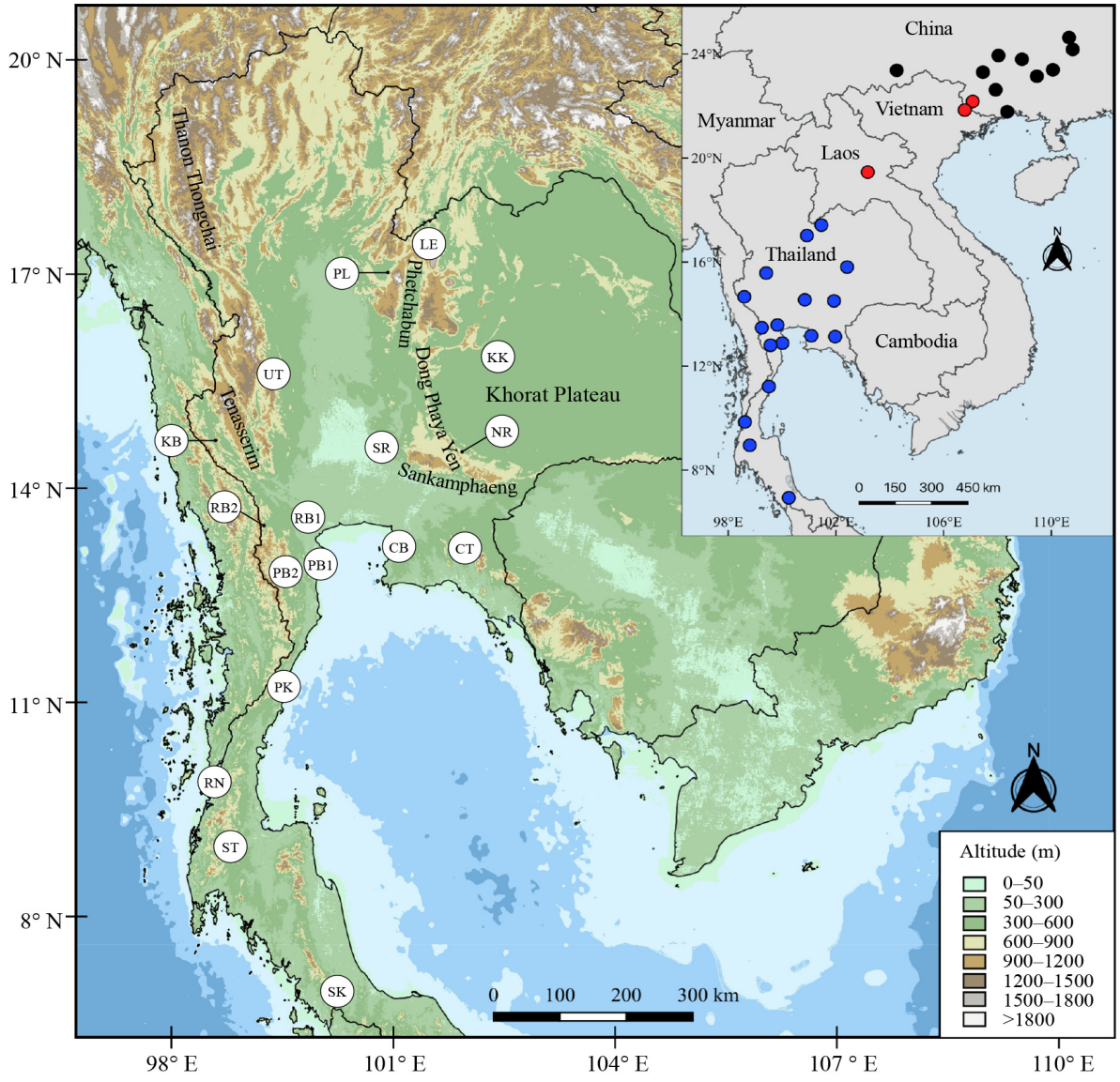

Figure 1 Map showing sampling localities in Thailand. The details of each locality are shown in Table 1. Blue dots indicate samples of *Gekko gecko* in Thailand. Red dots represent samples of *G. gecko* in Laos, Vietnam, and China. Black dots represent samples of *G. reevesii* in China.

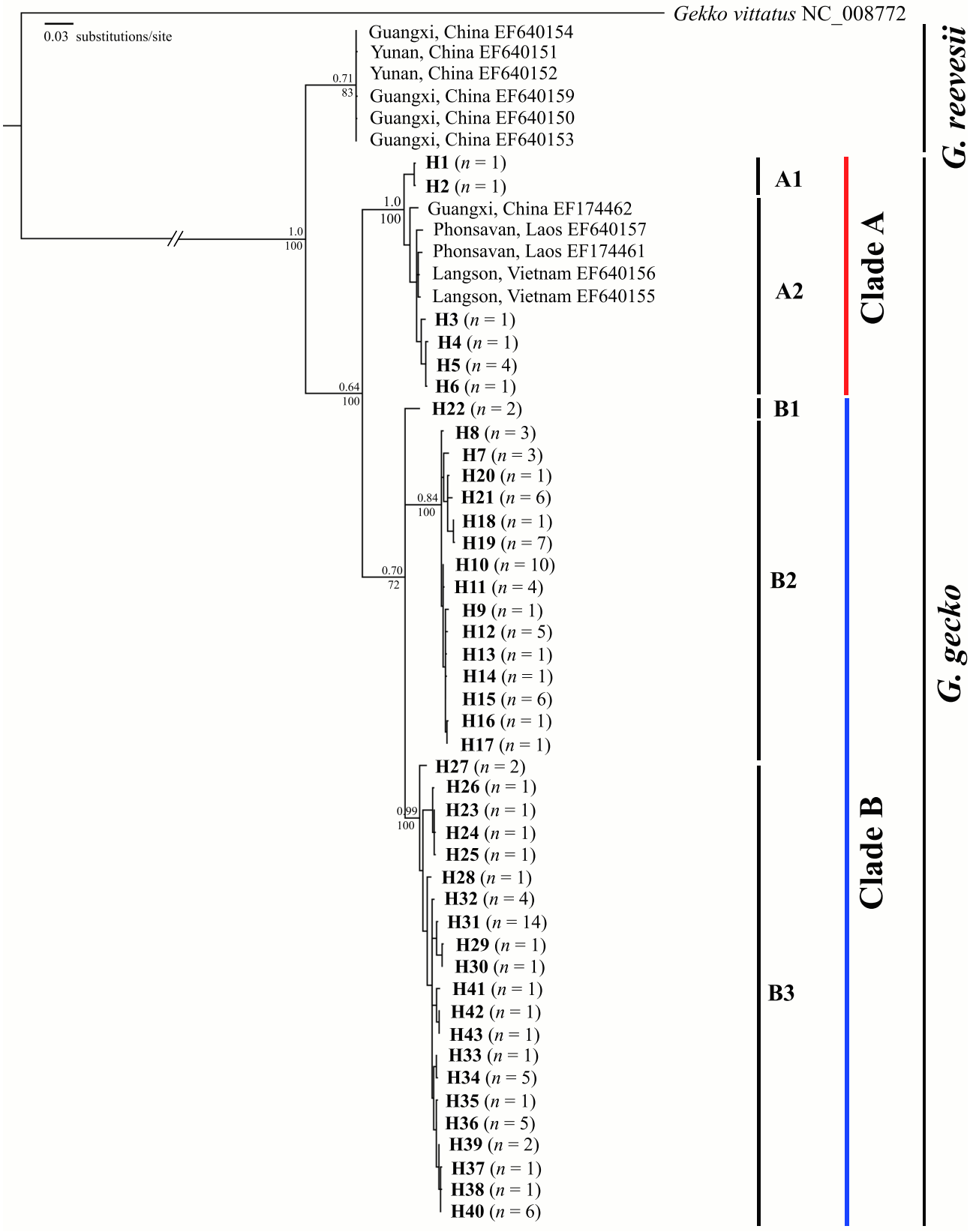

Figure 2 Phylogenetic tree, representing relationship among haplotypes and closely related species. Values above or below branch correspond to maximum likelihood bootstrap support and Bayesian posterior probability.

3. Results

3.1. Genetic diversity An alignment of 1 125 base pairs (bp) of cyt *b* sequences was obtained. There were 201 polymorphic sites, including 186 parsimony informative sites and 15 singleton variable sites. We identified 43 haplotypes within 114 individuals from 17 localities in Thailand. The haplotype diversity (*h*) and nucleotide diversity (π) of each locality are presented in Table 3. The overall haplotype diversity was high (0.960 ± 0.007), but nucleotide diversity was low (0.04076 ± 0.00253).

3.2 Phylogenetic analyses and haplotype relationships Phylogenetic analyses (BI and ML) of haplotypes showed similar tree topologies. *Gekko gecko* populations, formed a monophyletic group sister to *G. reevesii* . The BI tree (Figure 2) shows that *G. gecko* is separated into two major clades, A and B. Two subclades were recovered within clade A. Subclade A1 contained two individuals from PL and RB1, whereas subclade A2 consisted of individuals from NR, CT, PL, and SK, together with populations from Laos (Phonsavan), Vietnam (Langson), and China (Guangxi). Clade B comprised three subclades: B1 (ST population from southern Thailand), B2 (populations from northeastern and central (SR) Thailand, and two individuals from RB2), and B3 (populations from western and southern Thailand, and one individual from CB). However, the relationship among these three subclades was only moderately supported (BI posterior probability = 0.70, ML bootstrap = 72).

The median-joining network showed intraspecific genetic structure, comprising two major lineages (Clades A and B) within populations of *G. gecko* in Thailand (Figure 3) that were consistent with the phylogenetic tree topology. Most haplotypes were unique to one individual (60.47%). Five haplotypes (H5, H10, H11, H34, and H36) were shared among populations. Clade A was highly divergent from clade B and contained individuals from different regions in Thailand. Most individuals in clade A carried a unique haplotype, but H5 was shared by individuals from CT, NR, and PL. In subclade B2, H10 was the most abundant haplotype and occurred in geckos from three localities (NR, SB, and RB2), while H31 was the most abundant haplotype in subclade B3 and was unique to individuals from the PK population.

Table 3 Genetic diversity of each phylogenetic clade.

Subclade	Sample Size (*n*)	No. of haplotypes (*N*)	No. of unique haplotype (*Nu*)	Haplotype diversity (h ± SD)	Nucleotide diversity (π ± SD)
A1	2	2	2	1.000 ± 0.500	0.00178 ± 0.00089
A2	7	4	3	0.714 ± 0.181	0.00279 ± 0.00136
B1	2	1	1	–	–
B2	51	15	7	0.907 ± 0.018	0.00675 ± 0.00059
B3	52	21	14	0.899 ± 0.028	0.00971 ± 0.00097
All	114	43	26	0.960 ± 0.007	0.04076 ± 0.00253

Table 4 Uncorrected pairwise genetic divergence (%) between phylogenetic clades for mitochondrial cytochrome *b* dataset.

Clade	*Gekko reevesii* (*n* = 6)	A1 (*n* = 2)	A2 (*n* = 7)	B1 (*n* = 2)	B2 (*n* = 51)	B3 (*n* = 52)
Gekko reevesii	0.25 (0.09–0.53)					
A1	8.56 (8.36–8.80)	0.18 (0.18–0.18)				
A2	8.99 (8.71–9.33)	2.62 (2.58–2.76)	0.28 (0.00–0.89)			
B1	8.59 (8.44–8.80)	8.18 (8.18–8.18)	8.36 (8.27–8.44)	0.00		
B2	10.59 (10.13–11.29)	8.80 (8.44–9.33)	9.22 (8.80–10.04)	5.45 (5.07–6.04)	0.67 (0.00–1.33)	
B3	10.18 (9.60–10.93)	9.10 (8.80–9.60)	9.31 (8.98–9.78)	3.66 (3.29–4.09)	5.44 (4.71–6.13)	0.97 (0.00–2.58)

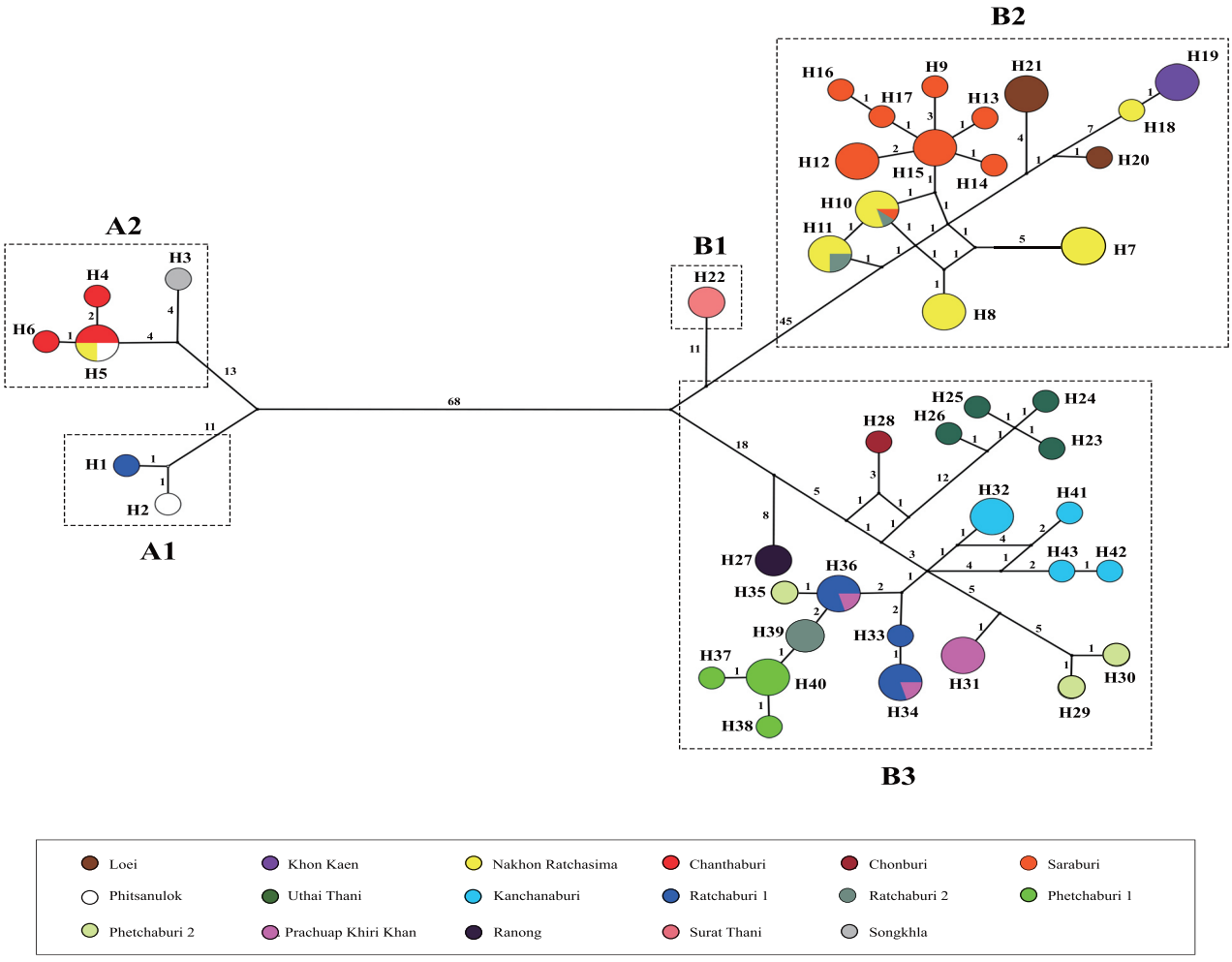

Figure 3 Median-joining network of mitochondrial cytochrome *b* haplotypes of *Gekko gecko*. The size of the circles is proportional to haplotype frequency. Each color represents locality.

Table 5 Pairwise F_{ST} (lower diagonal) and *P* (above diagonal) values of *Gekko gecko* mitochondrial cytochrome *b* dataset. Bold values represent significant F_{ST}.

Subclade	A1	A2	B1	B2	B3
A1	–	0.025	0.355	0.001	< 0.001
A2	**0.899**	–	0.013	0.001	< 0.001
B1	0.989	**0.971**	–	< 0.001	< 0.001
B2	**0.925**	**0.932**	**0.882**	–	< 0.001
B3	**0.897**	**0.906**	**0.755**	**0.849**	–

3.3 Population genetic structure and demography

Pairwise genetic distances (% *p*-distance) between clades are presented in Table 4. The results showed high mean genetic divergences between *G. reevesii* and *G. gecko* (8.56–10.59%). The mean *p*–distances between the major clades (Clades A and B) of *G. gecko* populations in Thailand were high (8.18–9.31%). The mean *p*–distances between populations within clade A were low (0.18–2.62%), whereas the mean *p*–distances between populations within clade B were higher than those within clade A (0.00–5.45%) and geographic structuring was

Table 6 Results of analysis of molecular variance (AMOVA) across and within major geographic regions of *G. gecko* based on mitochondrial cytochrome *b*.

Sources of Variation	*d.f.*	Sum of square	Variance component	Percentage of variation	Fixation index	*P*-value
Among groups	1	658.71	25.28	46.24	0.46	0.099
Among populations within groups	3	1451.23	24.99	45.72	0.85	< 0.001
Within populations	109	478.87	4.39	8.04	0.92	< 0.001

detected, with the presence of three subclades (B1–B3).

Overall mean pairwise F_{ST} varied from 0.755 to 0.925 and analyses revealed significant genetic differentiation between most subclades of *G. gecko* (Table 5). The high F_{ST} values indicated long-term restriction of gene flow between populations. AMOVA that revealed genetic differentiation among populations within group explained 45.72% of the total genetic variation, and 8.04% of the genetic differentiation occurred within populations (Table 6).

The mismatch distribution was relatively ragged and multimodal in each subclade (Figure 4; Table 7). These tendencies were also found in Fu's test of neutrality, showing non-significant values, whereas Tajima's *D* was significantly negative ($P = 0.011$) only in subclade A2, rejecting the hypothesis of constant population size. However, this group contained individuals from different regions in Thailand (CT, PL, NR, and SK).

4. Discussion

Whereas previous phylogenetic and phylogeographic studies of *G. gecko* have been conducted, they have included limited sampling from Thailand. This study carefully examined cyt *b* gene sequence diversity within and among 17 populations of *G. gecko* from residential areas in Thailand. Results of our analyses of partial cyt *b* gene sequences (1 125 bp) revealed two major mitochondrial clades (A and B) with high mean genetic divergence (8.18–9.31%). The mean genetic divergence within clade A ranged from 0.18 to 2.62% whereas that within clade B was higher, ranging from 0.00 to 5.45%, demonstrating that the genetic divergence among Thai populations in this study was much higher than those

Table 7 Results of statistical test of neutrality and mismatch distribution of sampled populations of *G. gecko* based on mitochondrial cytochrome *b*. Bold values represent significant value.

Clade	Tajima's *D*		Fu's Fs		Mismatch Distribution			
	D	*P*	Fs	*P*	SSD	*P*	*r*	*P*
A1[a]	–	–	–	–	–	–	–	–
A2	**–1.6236**	0.011	0.7516	0.644	**0.6259**	0.000	0.0907	1.000
B1[a]	–	–	–	–	–	–	–	–
B2	–0.08146	0.545	0.76685	0.691	0.01916	0.204	0.01921	0.544
B3	–0.75115	0.261	–0.50022	0.484	0.01611	0.164	0.02172	0.113

Note: [a] means the subclade less than three individuals were excluded from the population demographic analyses.

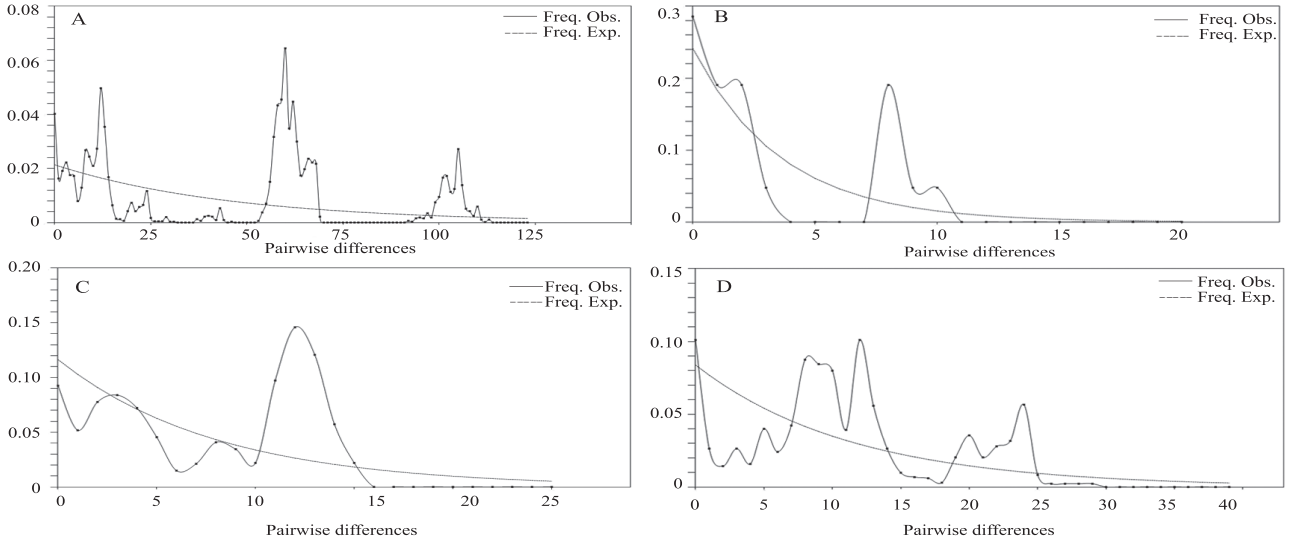

Figure 4 Observed frequencies of pairwise nucleotide differences among mitochondrial sequences (dashed lines) and expected frequencies under a model of sudden population expansion (solid lines). Mismatch distributions depict frequencies of pairwise differences for: (A) All subclades of *Gekko gecko*, (B) subclade A2, (C) subclade B2 and (D) subclade B3.

previously reported between populations of *G. gecko* from China (Guangxi), Laos, and Vietnam (0.5–2.2% (424 bp) in Qin *et al*., 2007; 0.12–1.66% (1 141 bp) in Qin *et al*., 2012). High levels of mitochondrial genetic divergence were reported in other vertebrates in Thailand such as *Hoplobatrachus rugulosus* (cyt *b*; Pansook *et al.*2012), *Chiromantis hasenae* (16S rRNA; Yodthong *et al*., 2014), and *Calotes mystaceus* (*COI*; Saijuntha *et al*., 2017), suggesting that strong genetic structuring of these species could resulted from geographic topography in Thailand and/or historical biogeographic process.

The results of the median joining network analysis (Figure 4) were concordant with those of the phylogenetic analysis of *G. gecko* populations in Thailand, showing two major clades (A and B) and genetic sub-structuring within each clade. Clade B was divided into three subclades, B1 (ST population), B2 (Northeast, Central, and two individuals from RB2), and B3 (West, South, and one individual from CB). Strong genetic differentiation (F_{ST}) indicated limited gene flow among the subclades of geckos (Table 5) that could have resulted from the presence of geographic barriers, such as mountain ranges, preventing dispersal and gene flow between subclades B2 and B3. The geographic topography of western Thailand contains steep hills and mountain ranges, i.e., Tenasserim and Thanon Thong Chai that run north-south orientation whereas the topography of northeastern Thailand contains plateau with lower elevations and mountain ranges, i.e., Phetchabun and Dong Paya Yen mountain ranges (Inger, 1999 ; Gupta, 2005). Therefore, these mountain ranges could play an important role as barriers of gene flow among *G. gecko* populations. A similar phylogeographic pattern was found in the rhacophorid, *Chiromantis hansenae*, with populations in northeastern Thailand separated from populations in western Thailand (Yodthong *et al*., 2014). These limited levels of gene flow could resulted from geographic barriers in western and northeastern Thailand and/or the limited dispersal ability of *C. hansenae*. In addition, the genetic structure in gecko populations could be affected by natural dispersal which is relatively limited in other geckos such as *Hesperoedura reticulata* and *Hemidactylus mabouia* (Hoehn *et al*., 2007; Short and Petren, 2011). Hoehn *et al*. (2007) suggested that a distance as small as 500 m is a barrier to the natural dispersal of *H. reticulata*. Short and Petren (2011) showed that genetic structure was found among populations of *H. mabouia* at a small regional scale and suggested that gene flow might be limited by both dispersal ability and geographic distance. Long-distance dispersal of some geckos e.g., *H. mabouia*, and *Hemidactylus brookii* has been reported in previous studies and could be explained by human-mediated transport (Short and Petren, 2011; Weterings and Vetter, 2018). Similarly, evidence of long-distance dispersal by human-mediated transport was revealed among populations of *G. gecko* in this study. For instance, H10 and H11 were shared by NR populations (northeastern Thailand), and RB2 population (western Thailand) whereas H5 was shared by CT (eastern Thailand), NR (northeastern Thailand), and PL (central Thailand) populations. Although the dispersal ability of *G. gecko* might affect the genetic structure of populations in Thailand, the dispersal pattern of this widely distributed species has not been studied. There is only a report on the foraging distance of *G. gecko* which ranges from 0.1–38.5 m from its retreat (Aowphol *et al*., 2006). Therefore, the dispersal patterns of *G. gecko* should be further investigated at the regional and landscape scales.

Kongbuntad *et al*. (2016) analyzed the population genetic structure of *G. gecko* from northern and northeastern Thailand, Laos, and Cambodia based on multilocus enzyme electrophoresis. They found that *G. gecko* populations in northeastern Thailand were more closely related to populations from Laos and Cambodia and suggest that the populations from the Khorat Plateau of northeastern Thailand might be separated from northern populations by the Dong Paya Yen mountain range. In other lizard such as *Calotes mystaceus*, the phylogeographic patterns from most regions in Thailand, excluding the southern region, exhibited two lineages: (I) northern, western and central regions, (II) northeastern and eastern regions; and this divergence may be due to the Phetchabun, Dong Paya Yen, and Sankambeng mountain ranges acting as barriers to gene flow (Saijuntha *et al*., 2017). These phylogeographic patterns are congruent with results from studies of other terrestrial organisms such as the centipede *Scolopendra dehaani* in mainland Southeast Asia (Siriwut *et al*., 2015). With these results and the findings of this study, sufficient evidence has been collected to suggest that intraspecific structure of terrestrial organisms in Thailand are primarily the results of historical biogeographic events in the region, namely the uplift of the Khorat Plateau (Racey, 2009).

Although the sampled populations of *G. gecko* in Thailand have a shared evolutionary history, a significant amount of time would be required for different populations to establish unique haplotypes, suggesting independent evolutionary histories. The unique haplotypes indicate limited gene flow among populations within clades A and B, despite this species being well-adapted to the human-mediated environment

and the lack of obvious geographic barriers between some populations. Interestingly, the genetic divergence within clade A was low (0.18–2.62%). The subclade A1 contained individuals from PL and RB2 whereas subclade A2 comprised populations from Laos, Vietnam, China, and eight individuals from NR, CT, PL, and SK. This finding indicates that *G. gecko* may have been introduced to different areas by anthropogenic activities such as commercial trade and human-mediated transport. In conservation assessments completed by the International Union for the Conservation of Nature (IUCN), *G. gecko* is listed as "least concern" because of its wide distribution, presumed large population and occurrence in natural and artificial environments; however, its population size and trends have not been evaluated (Lwin *et al*., 2019). International trafficking in geckos, especially *G. gecko* for both the pet trade and medicinal uses, occurs on a grand scale (Bauer, 2009). Lwin *et al*. (2019) suggested that international trade monitoring is necessary, including CITES monitoring to collect data on trade volumes. In conclusion, the results obtained through this study demonstrated substantial genetic differentiation among the *G. gecko* populations in Thailand, indicating limited gene flow that could be caused by the complexity of Thailand's topography and the dispersal ability of *G. gecko*. We recommend the extension of fine-scale sampling across the species' distribution ranges, combining morphological, and molecular investigations in future studies to facilitate understanding of the evolutionary history, and the demographic and taxonomic status of *G. gecko* (i.e., *G. g. gecko* and *G. g. azhari*). Additionally, this study provided evidence that human-mediated transport has affected the population genetic structure of *G. gecko* populations in mainland Southeast Asia (Thailand, Laos and Vietnam) and southern China. Therefore, conservation strategies should involve controlling the introduction pathways of non-native *G. gecko* populations such as commercial and pet trade routes to prevent further impacts on native populations and other species.

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